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Spermagglutination by Bacteria: Receptor-Specific Interactions

MANOJ MONGA* AND JAMES A. ROBERTS†

*From the *Department of Urology, Tulane University School of Medicine, New Orleans, Louisiana; and the †Tulane Regional Primate Research Center, Covington, Louisiana.*

ABSTRACT: The influence of genital infection on infertility has yet to be elucidated. We examined receptor–ligand interactions between sperm and *Escherichia coli* from patients with prostatitis. Two *E. coli* surface adhesins (P-fimbriae, type 1 fimbriae) and their specific receptor saccharides (α-galp-1-4-β-galp-*O*-methyl [gal-gal], mannose) were evaluated. Bacterial concentrations of 10^4 caused spermagglutination. P-fimbriae caused tail–tail spermagglutination that was inhibited by gal-gal. D-mannose concentrations are highest in the acrosomal region and type 1 fimbriae caused head–head agglutination that was inhibited by mannose. Strains with both fimbriae caused head–head and tail–tail agglutination that was inhibited by a mannose/gal-gal combination. *E. coli* agglutinated 40–75% of motile sperm. Seminal fluid provided 50–100% protection, with lower effectiveness against type 1 fimbriae. Understanding bacteria–spermatozoa interactions at the receptor–ligand level holds potential for treatment of infertility and development of spermagglutinating contraceptives.

Key words: Infertility, fimbriae, pili, *Escherichia coli.*

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Controversy and contradictions exist in the literature regarding the role of infection in infertility. The importance of the receptor–ligand interaction in the pathogenesis of urinary tract infection is well documented (Roberts, 1991, 1992). Certain *Escherichia coli* are pathogenic in the urinary tract because they possess fimbriae (pili), rigid filamentous proteinaceous appendages that attach to specific urothelial receptors. These receptors may be glycoproteins (as with type 1 fimbriae) or glycolipids (as with P-fimbriae). The essential receptor component in glycoproteins for type 1 fimbriae is an α-D mannose group (mannose). The essential minimal active moiety in glycolipids for P-fimbriae is an α-D-galp-1-4-β-D-galp (gal-gal). Fimbriae-dependent interactions can be confirmed if they are competitively inhibited by addition of the specific receptor component.

Because the surface of spermatozoa is rich in glycoproteins, even asymptomatic colonization of the male or female genitalia with Enterobacteriaceae may result in similar interactions. Isolation and characterization of the receptors may allow therapy aimed at prevention of colonization with spermagglutinating microorganisms, or directed at inhibiting the receptor–ligand interaction. The corollary would be development of monoclonally derived receptor clones capable of spermagglutination as a biological contraceptive. This study examines fimbriated strains of *E. coli* for evidence of receptor-dependent agglutination of spermatozoa.

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Correspondence to: Dr. James A. Roberts, Department of Urology, Tulane Regional Primate Research Center, 18703 Three Rivers Road, Covington, Louisiana 70433.

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Materials and Methods

Solutions

Tyrode's solution (0.8% NaCl, 0.0195% KCl, 0.0213% $MgCl_2$, 0.1015 $NaHCO_3$, and 0.01554% $CaCl_2$) was supplemented with 0.1% D-glucose and 0.3% bovine serum albumin (w/v). Receptor solutions consisted of α-galp-1-4-β-galp-*O*-methyl (2.2%, 60 mM), mannose (10%), and a combination of the two (2.2% gal-gal, 10% mannose).

Bacterial Samples

Midstream urine from males with clinical prostatitis was cultured. Eighteen strains of *E. coli* were subdivided into three groups based on the presence of P-fimbriae and/or type 1 fimbriae. Bacterial isolates from blood agar plates were resuspended in 0.9% NaCl.

Sperm Samples

Sperm samples were obtained from a healthy volunteer by masturbation, following a 24-hour continence period, into a sterile wide-mouth beaker. Experiments were performed within 1 hour of obtaining the sample. Samples underwent liquefaction at room temperature for 30 minutes. Ejaculate was washed twice with Tyrode's solution (1:1, v/v) and centrifuged at 300 × *g* for 10 minutes. Initial supernatant was saved as seminal fluid. The final cell pellet was resuspended in Tyrode's solution to a final concentration of 5×10^6.

Sperm–bacteria Interactions

Sperm and bacteria (1:1, v/v) were incubated at 37°C for 15 minutes, then examined using light microscopy and scanning

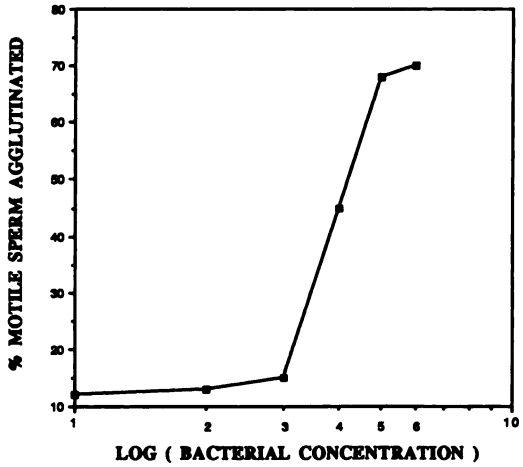

FIG. 1. Concentration-dependent agglutination of motile sperm by *E. coli*: threshold of 10^4 bacteria/ml required for significant agglutination.

electron microscopy. All studies were done in triplicate and the results expressed as percent adherence plus or minus the standard deviation. Control consisted of Tyrode's solution instead of bacteria. Samples were filtered by gravity through glass wool columns (20 mg of glass wool fiber loosely distributed in a disposable 5-inch glass Pasteur pipette) to remove the agglutinated component (Paulson and Polakoski, 1977a). The number of motile sperm in the effluent was counted, and the percentage of motile sperm agglutinated was calculated using the Tyrode's solution control as a baseline. Incubations were repeated with the addition of seminal fluid, mannose (specific receptor minimal moiety for type 1 fimbriae), gal-gal (specific receptor minimal moiety for P-fimbriae), or the mannose/gal-gal combination to the bacteria 15 minutes prior to incubation with the washed sperm.

Results

A threshold concentration of 10^4 bacteria was required to obtain significant spermagglutination (Fig. 1). P-fimbriated strains caused a tail–tail agglutination (Fig. 2) of 48 ± 1.8% of the motile sperm that were partially inhibited by the addition of seminal fluid (14 ± 1.6%) or gal-gal (16 ± 1.6%) to the bacteria prior to incubation with washed sperm (Fig. 3). Type-1 fimbriated strains caused a head–head agglutination (Figs. 4, 5) of 57 ± 2.2% of the motile

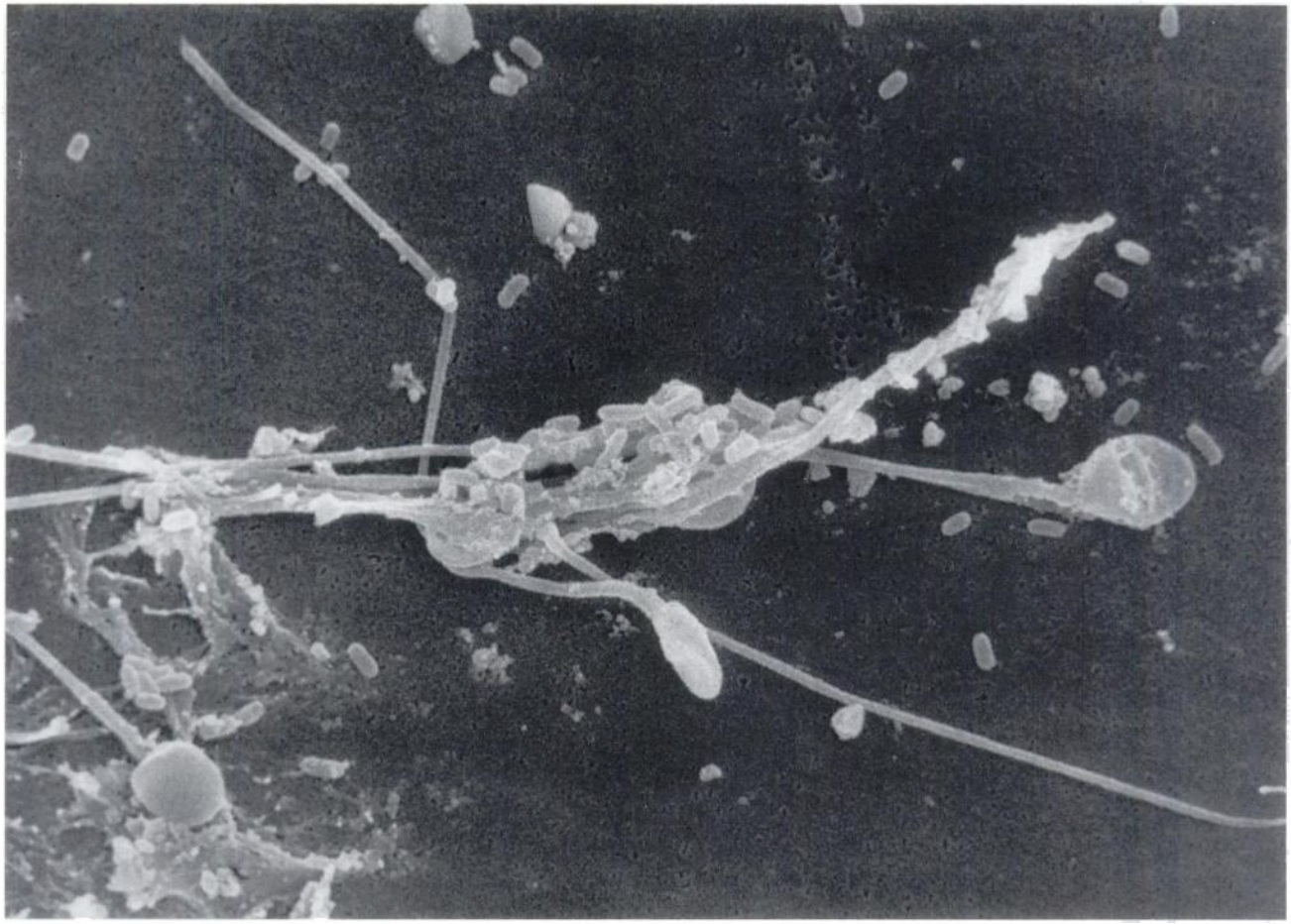

FIG. 2. Tail–tail agglutination by P-fimbriated *E. coli*. P-fimbriated *E. coli* caused a tail–tail agglutination of 48% of the motile sperm. This was partially inhibited by the specific receptor for P-fimbriae, gal-gal, and by seminal fluid (6,000×).

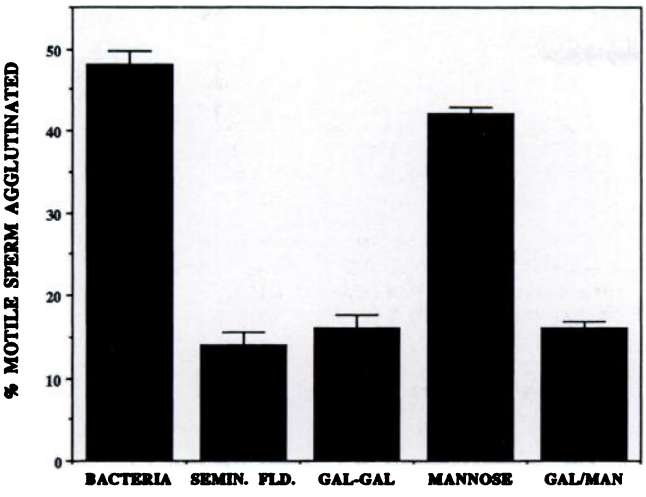

FIG. 3. Agglutination of motile sperm by P-fimbriated strains of *E. coli*: partial inhibition of agglutination by the addition of seminal fluid and gal-gal.

sperm that were partially inhibited by the initial addition of seminal fluid (32 ± 1.3%) or mannose (12 ± 1.0%) (Fig. 6). *E. coli* strains with both type 1 and P-fimbriae caused a combination of head–head, tail–tail, and head–tail (Fig. 7) agglutination of 69 ± 3.6% of the motile sperm that were inhibited by addition of a combination of both specific receptors, mannose and gal-gal (20 ± 1.2%) (Fig. 8).

Discussion

Previous studies have evaluated the ability of bacteria to affect sperm motility by adherence, agglutination, and dialyzable factors; however, none have identified a receptor–ligand interaction between spermatozoa and bacteria obtained from patients with prostatitis. We have shown this same interaction using an *E. coli* strain obtained from a vaginal culture of an infertile woman (Lewis RW and Roberts JA, unpublished).

The instant and irreversible spermagglutinating ability of *E. coli* was first demonstrated in 1931, suggesting a role for vaginal and cervical bacteria in infertility (Rosenthal, 1931). Teague et al (1971) demonstrated similar results using a bacterial concentration of 10^{13} *E. coli*/ml. Del Porto et al (1975) reported decreased motility with

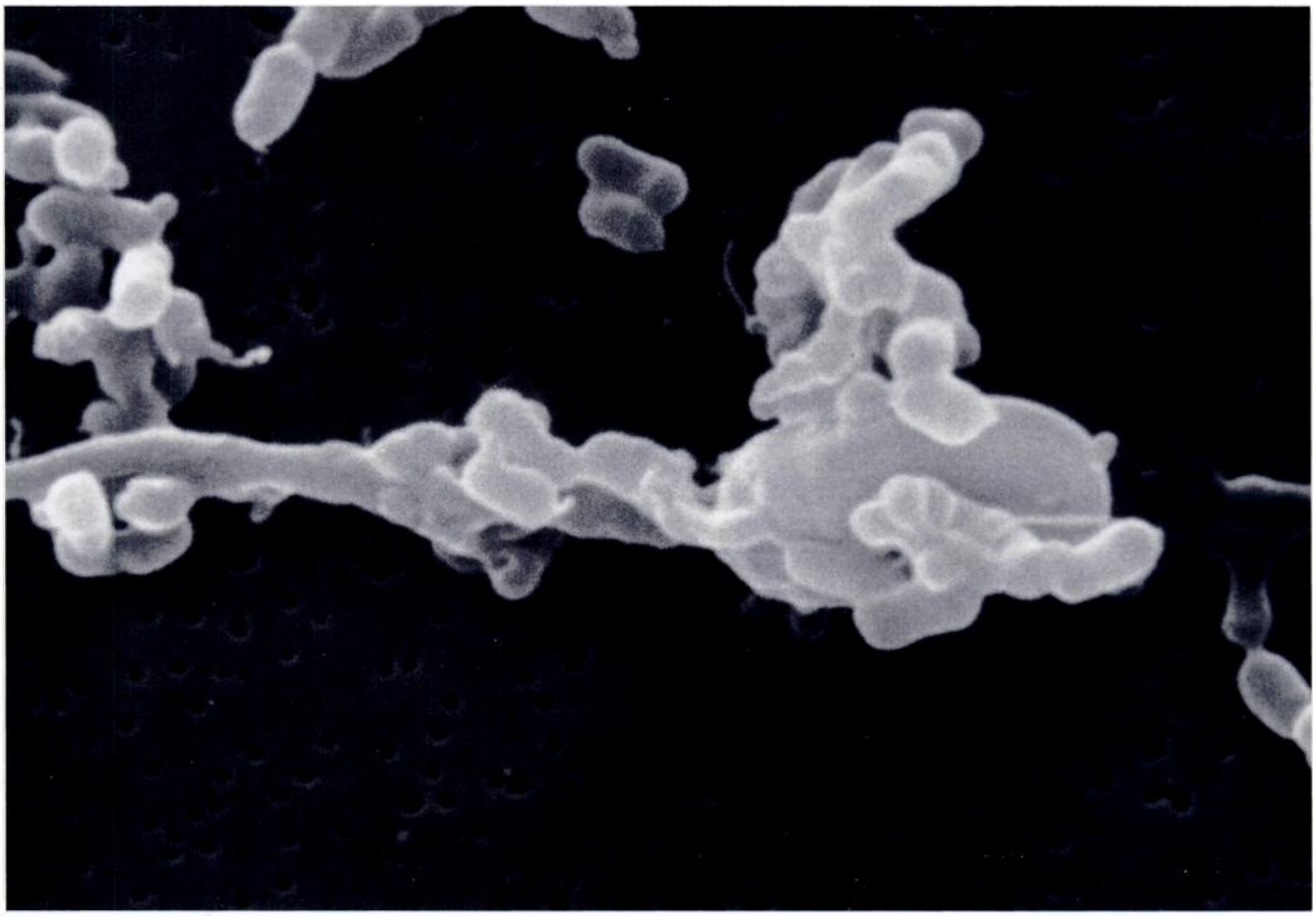

FIG. 4. Adherence of type 1 fimbriated *E. coli* to spermatozoa (15,000×).

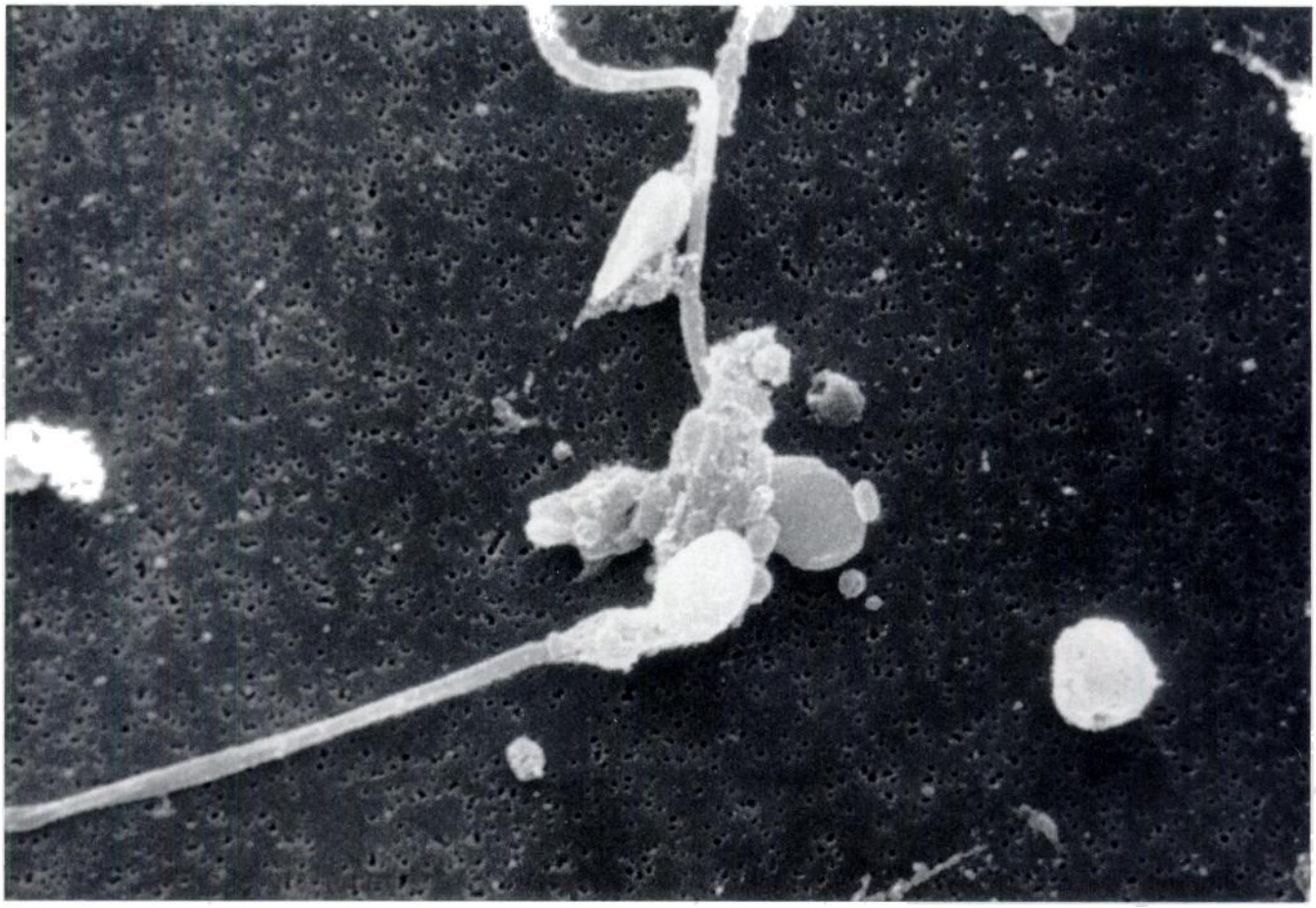

FIG. 5. Head–head spermagglutination by type 1 fimbriated *E. coli*. Type 1 fimbriated *E. coli* caused a head–head agglutination of 57% of motile sperm. This was partially inhibited by the specific receptor for type 1 fimbriae, mannose, and by seminal fluid (6,000×).

concentrations of 10^6 and agglutination with 10^7 *E. coli*/ml. Paulson and Polakoski (1977b) isolated a heat- and cold-resistant dialyzable spermatozoal immobilization factor from the filtrate of *E. coli* suspensions. Matthews and Buxton (1951) demonstrated decreased sperm motility and agglutination of sperm when they were incubated with *E. coli* obtained from cervical cultures of infertile females. These effects were partially inhibited by the addition of seminal fluid and the bactericidal agent, streptomycin.

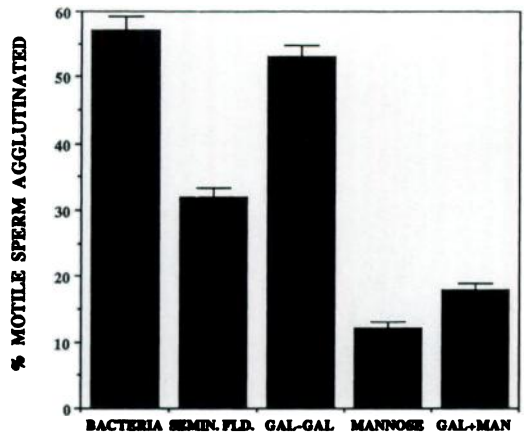

FIG. 6. Agglutination of motile sperm by type 1 fimbriated strains of *E. coli*: partial inhibition of agglutination by the addition of seminal fluid and mannose.

Random adherence of *Chlamydia trachomatis* to spermatozoa has been demonstrated by immunofluorescence and transmission electron microscopy. Adherence was favored with increasing chlamydial concentrations and acidic pH, similar to that in the posterior vaginal vault (Wolner-Hanssen and Mardh, 1984).

Mycoplasma species have been demonstrated to adhere to and agglutinate sperm (Taylor-Robinson and Manchee, 1967; Busolo et al, 1984). Spermagglutination by myxovirus was inhibited by virus-specific hyperimmune sera and by seminal plasma (Peleg and Ianconescu, 1966). Spermagglutination by *Candida albicans* was demonstrated at 4 hours with concentrations of 10^4 organisms or greater (Tuttle et al, 1977a). Decreased spermatozoal motility was demonstrated when incubated with 10^6 *Trichomonas vaginalis* (Tuttle et al, 1977b). Bacterial adher-

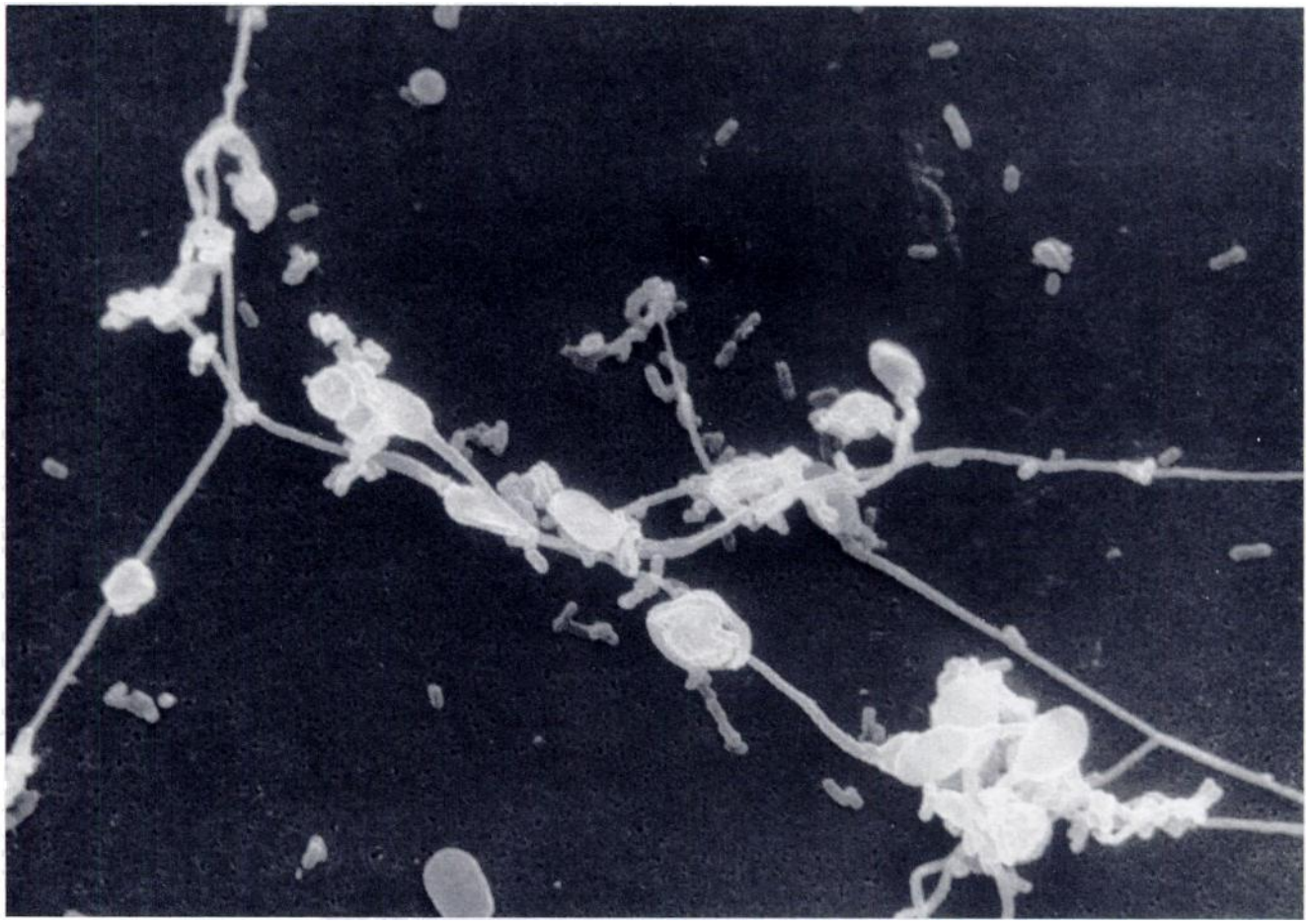

FIG. 7. Spermagglutination by P+, type 1+ *E. coli*. P+, type 1+ *E. coli* caused a combination of head–head, head–tail, and tail–tail agglutination of 64% of motile sperm. This was partially inhibited by a mixture of both specific fimbrial receptors: mannose and gal-gal (5,000×).

ence to sperm has been suggested to play a role in ascending route to epididymitis and salpingo-oophoritis (Howard, 1971; Toth et al, 1982). One investigation reported adherence of *Neisseria gonorrhoeae* to spermatozoa that was enhanced with fimbriated strains and inhibited by anti-fimbrial antibodies; however, adherence was not exclusive to fimbriated strains (James-Holmquest et al, 1974).

This study identifies a receptor–ligand interaction between fimbriated *E. coli* and spermatozoa that results in spermagglutination and significant decreases in motile sperm count. Bacterial concentrations of 10^4/ml caused spermagglutination. The concentration of bacteria in a typical prostate infection is up to 10^5/ml (Hellstrom and Neal, 1992); thus, an ejaculate during prostatitis would deposit more than the concentration that we found would cause agglutination. Earlier studies by Stamey et al (1971) of women with recurrent urinary tract infections showed that $>10^4$/ml enterobacteria were often found prior to or during a urinary tract infection.

Because the surface of spermatozoa is rich in glycoproteins, even asymptomatic colonization of the male or female genitalia with fimbriated bacteria may result in similar species-specific interactions, causing agglutination of motile sperm. Lectin-probes for terminal saccharides on the sperm surface in animal studies have demonstrated a high concentration of α-D-mannose on the head and midpiece; however, such distributions may be species specific (Edelman and Millette, 1971; Benoff et al, unpublished data).

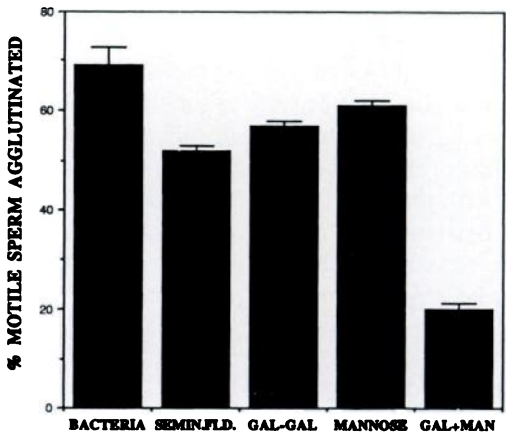

FIG. 8. Agglutination of motile sperm by type 1 and P-fimbriated strains of *E. coli*: partial inhibition of agglutination by the addition of a combination of mannose and gal-gal.

The role of the mannose-acceptor on the head of spermatozoa in conception and in antibody-mediated infertility has been recently studied. The mannose-specific sperm membrane lectin receptor for zona ZP-3 oligosaccharide plays a putative role in human sperm–egg recognition. Mannose-specific lectin binding by fresh and capacitated sperm was markedly inhibited by female sera containing high levels of sperm-head-directed IgG and IgA (Benoff et al, unpublished data). Sperm that failed to fertilize eggs *in vitro* in spite of normal morphology, concentration, and motility were shown to bind significantly less mannose-conjugated albumin in response to capacitating conditions (Benoff et al, 1993, unpublished data). Homology between this sperm membrane lectin and human macrophage mannose receptor lectin has been demonstrated by northern hybridization (Benoff et al, unpublished data). Sensitization of macrophage to bacterial type-1 fimbriae may elicit a lineage-specific macrophage colony-stimulating factor (M-CSF, CSF-1) that directs the phenotypic differentiation of macrophage progenitor cells, similar to the effect of lipopolysaccharide stimulation (Neta et al, 1990; Burd et al, 1992). Head–head spermagglutination that could be inhibited by D-mannose has been demonstrated using monoclonal antibodies directed at carbohydrate epitopes on a purified abnormal mucin secreted by men with cystic fibrosis (D'Cruz et al, unpublished data).

This study identifies a receptor–ligand interaction between *E. coli* and spermatozoa that results in spermagglutination. Clinical studies to examine the prevalence of similar interactions in fertile and infertile couples are needed. It is possible that factors in infertile individuals may influence phase variation of expression of type-1 and P-fimbriae, causing non-fimbriated *E. coli* to undergo phenotypic change to the fimbriated state (Roberts, 1992). Host receptor density on the surface of spermatozoa may affect the susceptibility of the individual to spermagglutination. Proteins in the seminal fluid may bind to and mask terminal saccharides on the sperm surface. It is possible that protective effects of seminal, vaginal, and cervical secretions and the vaginal mucosa may be dependent on the secretor state of the individual, where A, B, O, P, or Lewis blood group oligosaccharides in the secretions or on the mucosal cells of secretors would interfere with the receptor–ligand interaction (Berger et al, 1983). Systemic antibiotic therapy against agglutinating bacteria or intravaginal therapy using anti-pili antibodies may be of benefit in improving fecundity.

Acknowledgments

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